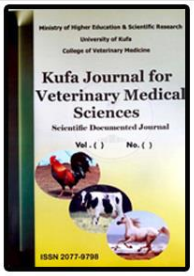
Kufa journal for Veterinary Medical Sciences Vol.(10) No.(2)

Kufa journal for Veterinary Medical Sciences

Kj.vs@uokufa .edu.iq

www.uokufa .edu.iq\journals\index.php\kjvs



Necrotic Enteritis In Poultry: A Scientific Review

Mohammad. A. Hamad

Department of Microbiology / College of Veterinary Medicine- University of Mosul

Mahmah1073@gmail.com

ORCID number: https://orcid.org/0000-0001-9278-5391

Received date:8Dec.2019 **Accepted:(463B)** 8 Dec.2019 **page** (82-93) **Pubished:**31Dec 2019

Abstract:

Necrotic Enteritis (NE) is a disease of domestic chicken and a worldwide distribution. The disease also occurs in turkey flocks, domestic ducks, and some kinds of wild birds like wild geese, wild ducks, crows, and ostrich and numerous kinds of caged wild birds. The disease usually caused by *Clostridium perfringens* type A or type C and in some cases, isolates of type D and untyped strains were also mentioned. The disease hapen as keen clinical illness necrotic enteritis, in chicken about 2-4 weeks of age, producing extreme death-rate, and also as an unrealizable illness with centric necrosis in the bowel or as C. *perfringens*-connected hepatitic alteration (CPH) with cholangiohepatitis or fibrinoid necrosis in the liver.

Key words: *Clostridium perfringens*, Necrotic enteritis, Chickens.

التهاب الأمعاء النخري في الدواجن . مراجعة علمية

أ.م.د. محمد علي حمد

فرع الاحياء المجهرية/ كلية الطب البيطري / جامعة الموصل

الخلاصة:

مرض التهاب الأمعاء النخري هو مرض شائع في الدجاج المستأنس وهو من الأمراض المنتشرة عالميا. المرض يحدث أيضا في قطعان الديك الرومي ، والبط المنزلي و بعض أنواع الطيور البرية مثل الإوز البري، البط البري، الغربان، النعام، وفي بعض الطيور البرية المرباة في الأقفاص. المرض مسبب بمطثية بيرفرنجنز نمط A أو نمط C، و في بعض الاحيان عزلات للنمط D و عزلات غير منمطة أيضا سجلت من حالات لهذا المرض. المرض يحدث كمرض إكلينيكي حاد، هو التهاب الأمعاء النخري (NE)، في الدجاج بعمر 2-4 أسبوع، يسبب نفوق عالي،ويحدث أيضاً كمرض تحت إكلينيكي مع نخر بؤري في الأمعاء أو كإصابة بمطثية بيرفرنجنز مترافقة مع تغيرات كبدية (CPH) و التهاب الاقنية الصفراوية والكبد أو نخر شبيه الفبرين fibrinoid في الكبد.

الكلمات المفتاحية: مطثية بيرفرنجنز، التهاب الامعاء النخري، الدجاج .

Introduction:

The poultry industry has ripe intensely during the last decades, converted oneself into a robustly specialized scope that requires fundamental economical investment (1). Enhanscement in habitation and field tools have procured to spectacular excesses in providing intensity, and heriditrial selection has led to cursory growing, better food divertion , greater meat production, and minimize mortality. The increase in means growing and food adequacy partly recompense for approximately 65-70% of

overall producer expenses that invest in the feed. On the other hand, discrepancy in winning and loss is allmost determined by the occurrence of several contagious illnesses that can infect poultry. Necrotic Enteritis (NE) represent one of them , which causes the loss of the global poultry industry about 2 billion dollars yearly (2-3). The delayed growth and a rising death-rate affect principally on the profitableness of poultry production (4-5), and necrotic enteritis forms (Peracute and Acute) is the principal clinical bacterial illness in poultry (4).

Necrotic enteritis is a worldwide spreading disease of the domestic chickens (6-9). The disease is also present in turkey herds (10), domestic ducks and some wild birds such as brutal goose (11), brutal ducks (12), crow (13), ostrich (14), and some wild birds nurtured in cages (15).

The scientist Bennetts in 1930 isolated *Clostridium perfringens* from gut infection in black Orpington chicken and the mortalities of birds were due to it (cited by 16). The disease was then called the six-day disease by the scientist Mann in 1945, which was characterized by the invasion of the *C. perfringens* to the intestine of chicks (cited by 17). This condition has been re-established in one-day-old chicks by giving the bacterium orally (17).

The term necrotic enteritis was first used in the 1960s to describe intestinal disorders in small cocks by the reseacher Parish and he reintroduced it experimentally (18-20). Clinical attacks of NE in broiler mentioned frequently in many countries, and it causes serious problems, and the disease predominantly prevailing in countries with bushy broiler production (21).

Clinical NE is predominantly visible in broilers, but it may also be appear in the youth breeders broiler and layers that raise on a straw litter; or a litter contaminated with feces (12)

The causative agent:

Clostridium perfringens type A is the causative agent of NE (8-9, 22-24) or type C (25-26), and occasionally type D isolates (27) and also non-typed isolates have been recorded in cases of the disease (8, 28-29). *C. perfringens* is the utmost remarkable species of Clostridia; which today associated with most health problems in poultry, where it cause arsenal diseases including necrotic enteritis, avian malignant disease, Gizzard erosions, and gangrenous dermatitis in chicken (12, 30-35).

C. perfringens type A is common in the gut of vertebrates (Including poultry) (4, 26, 32, 36), soil, dust, contaminated food and waste, which are considered as sources of infection (32, 37). The disease can be reintroduced by raising chicks on the waste of buildings that have past with disease (8, 30-31) or by giving contaminated food (32, 38) or cultures of *C. perfringens* (23, 39), or floating fluids of bacterial cultivation (40).

Both alpha (α) and beta (β) toxins were appeared in faeces and gut fillings of broiler and others with this disease (28, 36, 41), and it assume that they are responsible for gut necrosis, which distinguishes the disease (42). Alpha-toxin may be product by bacterial strains of birds infected with the disease in largeer amounts than bacterial strains of natural birds (15) and is thoughted to have a prme part in the occurrence of the disease (15, 43). Alpha-antitoxin perhabs playing a part in the prophylaxis of birds from enteritis (44). It was observed that immunizing by alpha-toxoid produced significant protection against NE (17). NetB toxin is a newly described toxin and is believed to be implicated in the pathogenesis of infection in many strains of *C. perfringens* that cause the disease (45-46), and in very recent studies (47-48) they attribute that this toxin play the main part in the pathogenesis of *C. perfringens*.

C. perfringens isolates are Gram-positive bacteria, rod -shape and has a semi-terminal

ovoid spore, which differs from many of the clostridia that are comparatively big bacilli (0.6- 2.4 x 1.3-9µ), capsulated and non-motile. Their colonies are circular, glistening, smooth, surrounded by two zones of blood hemolysis. A narrow internal district of entire blood hemolysis produced by theta toxin and an external district of partial hemolysis produced by α-toxin. The bacterium grows anaerobically, although it sometimes lives and grows with the existence of oxygen (49-50). growing is happen within temperatures of (12-50°C) but so tardily in temprature below 20°C (51). In normal states (43-47°C), these bacteria growing up very fast with a generation time of about 8-10 minutes, their Growing is escort by profuse gas production (50). This microorganism needs 13 principals amino acids for growing (36, 52). These bacteria are grown in pH ranging from 5-8. Bacteria can live under extreme conditions, where they are transformed from vegetative cells into high-resistance endospores (36, 50). *C. perfringens* is dividing into main five types (A-E) depending on production of the four principal toxins, which are Alpha (α), Beta (β), Epsilon (ε), and Iota (ι) (15, 49, 53-54).

Predisposing factors of Necrotic enteritis:

Clostridium perfringens is mainly present in the gut of normal birds, which can produce the disease when the predisposing factors are present. These factors include high-fiber feed that can injury the gut mucosa, so the broiler will develop NE (17, 39, 55), and infection can coincide with coccidiosis, particularly *Eimeria maxima* and *Eimeria acervulina* (17, 26, 39, 41, 55, 56).

Johansson and Sarles in 1948 referred that intestinal mucosa is damaged by sporozoites and merozoites (cited by 17). This effect with pH declining of the intestine (39, 57) will facilitate colonization and multiplication of *C. perfringens* (56-59). It was also noted that *C. perfringens* number in the gut and cecum of birds infected with acute coccidiosis is many times greater than the normal number, and these bacteria attached with high rates on cecal mucosa of specific pathogen free chicks when infecting with the *Eimeria* in comparing with uninfected chicks (55, 58). The death-rate in broilers which have co-infection with *C. perfringens* and *Eimeria* spp. are basicly 25% higher at least than birds free from *Eimeria*)60).

The food has apparent effects on NE incidence in chickens (55, 61-62), of these effects, food may contain high content of undigested water dissolved polysaccharides, e.g. rye, barley and grist (63-64), may direct to rise in the number of *C. perfringens* in the gut (32, 64). It was observed that the birds that feed on the high-energy and protein-rich diet, such as wheat, barley, fish meal, or other animal proteins, is at least ten times more likely to be infected with NE than yellow corn-based food (17, 65-67), and the mortality is 2-3 times higher (68), also zinc additives into feed increase the rate of attacks in birds challenged with *Eimeria* and *C. perfringens* (69-70).

Intestinal flora may also affect the happening and acuteness of the disease (64, 71), where observed that when *lactobacillus acidophilus* or *Enterococcus faecalis* were given to chicks and then infected with C. perfringens; the birds have not died, in comparing with rate of mortality about 50% in chicks free of germs. In addition, the co-culture of *C. perfringens* with the contents of the chick's intestines has inhibited or stopped the production of alpha-toxin (67, 71-72).

Forms of the disease:

The disease occurs as an acute or peracute obvious illness called necrotic enteritis (NE); in chickens aged 2-4 weeks producing considerable deaths, and as well it occurs as a subclinical illness with focal intestinal

necrosis or as C. perfringens infection accompanied by hepatic pathological changes (HPC) with cholangiohepatitis or semi- fibrinous necrosis within the liver (8, 12). In a small layer, the disease was observed in crop (73) and was recorded as a case related to infection with *C. perfringens*, also it has cultivated from similar lesions in the crop of broiler and Turkey (12).

Sources of Infection:

The source of *C. perfringens* strains that causing necrotic enteritis is the chicks themselves. Possibly few strains permanently or transiently inhabit the normal chicken intestines (17). Chicks are acquired these strains from the hatching environment (68, 74), so from normal shedding or through subclinical attacks the contamination of growing environment will occur. Colonizing additional birds , pollution of the bedding and other aspects of the environment will increase the number of these bacteria (9, 17, 70).

Pathogenesis, symptoms , and necropsy findings:

The incidence of necrotic enteritis is related to the propagation of *C. perfringens* in the gut then following by an increase in the production of the toxin. In dead and clinically diseased birds with necrotic enteritis, abundant numbers of *C. perfringens* might be appeared in about 10^7-10^{10} colony/ g of intestinal or mucosal contents (59, 75), While in healthy birds the bacterial population is between 0 - 10^5/ g of gut fillings and is considered natural (75).

The source of the infection is the *C. perfringens* type A in territory, dust, tainted food and waste (74). A numerous of researchers confirmed that alpha-toxin is the most important in pathogenicity of NE (22-23), although there are other toxins that may be produced by the bacteria in the diseased cases. Where they produced typical lesions by using native alpha-toxin from type –A strains, and observed the disappearance of pathological changes when they used the toxoid.

The symptoms of NE are analogous to the most poultry illnesses (17, 76). Acutely infected birds show lethargy, loss of appetite (21, 33), motion impediment, drooping wings and head, and feathers ruffling (4, 21, 68, 77), and somnolent (39).

Diarrhea also occurs (68). Birds often become dehydrated and smelly and the disease is oftentimes peracute with doom within 1-2 hours (33, 78). Mortality rates range from zero to fifty percent (26, 79).

The obvious macroscopic pathological outcomes are limited to the jejunum and ileum (4, 21, 68), but lesions may befall in other parts like the cecum and duodenum (68), liver and kidney (8). The duodenum, jejunum, and ileum are fluffy in-wall and gas accumulated (63, 68, 80).

The spreading necrosis of mucosa in big portions of the small intestine is enveloped with a pseudo-yellow-brown or yellow-pigmented membrane that is the predominant lesion of acute necrotic enteritis. This membrane is composed of necrotic intestinal villi, degenerative epithelial cells and trapped- fibrin inflammatory cells (21, 77, 81), with huge gram-positive bacilli that are accompanied by necrotic regions but are not attack living cells (4).

C. perfringens bacteria are inter in the circulation and bile ducts, which is causing cholangiohepatitis (82). Besides the severe type of the illness, there is usually a description for the mild sub-clinical form seen in the field, steering to a decrease in production. In those circumstances, sores appear as Subsides into the surface of the mucosa or pseudomembrane, with a non-distinguished substance attached to the gut surface (63).

Diagnosis:

The diagnosis of NE depend mainly on case history, clinical signs and necropsy findings,

staining of direct slides from the mucosa of the upper part of the intestine using Gram's stain that must show a considerable or high numbers of large Gram-positive rods recognizes as Clostridium, then trying to isolation of *C. perfringens* (50, 83-85).

There are many artificial media that use for cultivation of *C. perfringens* including Clostridial agar, C.W. agar (*Clostridium welchii* agar), Thioglycolate medium, Blood agar, Brain heart infusion broth, SPS (Sulphite-Polymyxin -Sulfadiazine) SFP (Shahidi –Ferguson Perfringens)(50, 84) .

The culturing must be done under anaerobic or microaerophilic conditions using anaerobic cabinet or gas pack, and incubate the cultures at 37°C for 24-48hs (50,84-85).

The most famous biochemical tests that using for characterization of *C. perfringens* with their results are include sugars fermentation (Glucose, sucrose, lactose, maltose), negative oxidase-catalase, negative indole, positive lecithinase activity on egg yolk agar producing opalescent change or clear zone around growing colonies, stormy fermentation reaction in litmus milk medium (50, 86). In present-day, there are many miniaturized commercial systems like API20A (Analytab products), ATB32A (BioMe´rieux). (87)

Typing of *C. perfringens* from growing isolates or directly from intestinal content is carrying out by many techniques or tests including toxin neutralization test, ELISA, and PCR (46,50, 88-92). In the last decades, multiplex-PCR developed and it facilitated the typing of *C. perfringens* isolates in short times and in one reaction instead of separate reaction for each type on the isolate itself (48 ,90, 92-93).

Prophylaxis and treatment of Necrotic enteritis:

NE may be prevented or suppressed by constricting exposition to hazard agents such as Eimeria infection and inaproperate food (78, 94). The change in food composition, such as the removal of fishmeal from the feed, is active in reduction the occurence of the disease (21). Using antibiotic growth promoters (AGPs) in broiler and other birds feed is popular. However, there are great afraid that pathogenic bacteria in animal foods may transform antibiotic resistance to human pathogens (5), and the consumer is influenced by medicines reminant in the feed (95).

The European federation prohibited the use of AGPs (68), so as a positive result, there was a decrease in the spread of antibiotic-resistant bacteria in animal feed (8), While the negative effect appeared as a rising in the occurrence of NE, especially in Western Europe (2, 68).

NE is treated orally through drinking water using lincomycin, bacitracin, oxytetracycline, penicillin, or tylosin. Also, it can use some of these antibiotics with feed (21, 30-31, 57, 79).

Competing for excluding to preventing NE also studied. There is obvious worth for the building of microbiota (6, 9, 72), but it delays the reproduction of C. perfringens and decreases the occurrence of the disease only within the first month of life (65). The use of probiotics like *Streptococcus faecium* and *Lactobacillus acidophilus* may reduce the severity of NE (21, 68), and stimulates the growth of beneficial intestinal flora (9, 51, 68), where observed that when *lactobacillus acidophilus* or *Enterococcus faecalis* were given to chicks and then infected with C. perfringens; the birds have not died, in comparing with rate of mortality about 50% in chicks free of germs. In addition, the co-culture of *C. perfringens* with the contents of the chick's intestines has inhibited or stopped the production of alpha-toxin (71-72, 96-97).

Vaccination:

Vaccination of breeder broiler with Toxoid of A and C types; produces a strong immune response (IgY) for type A (34, 98). The

antibodies are transmitted (equal to those in the breeder) to offspring. safeguard of chicks from this disease and liver infectio was greater in chicks immunized with toxoid type C (34).

Conclusions:

- NE is a complicated illness that is high noticeable to the mercantile broiler industry due to the econimically expenses related to affected herd.
- Distinguishing the means that induce C. perfringens overgrowing in the gut of birds and produce the disease is necessary for domination of this disease.
- Reduction of influences that induce proliferation of these bacteria is one of the main principles for controlling NE disease.
- NE stays a defy for the broiler industry, and this defy is beginning to be bigger every once, with extra rigorous rules and forwarding humans to the products that produce with minimized AGP.

References:

1. Chapman, H.D., Johnson, Z.B., McFarland, J.L. (2003). Improvements in the performance of commercial broilers in the USA: analysis for the years 1997 to 2001. Poult. Sci.; 82(1):50–3.
2. Sluis, V.W. (2000). Clostridial enteritis is an often-underestimated problem. World's Poult. Sci. J. 16:42–43.
3. McReynolds, J.L., Byrd, J.A., Anderson, R.C., Moore, R.W., Edrington, T.S., Genovese, K.J. (2004). Evaluation of immunosuppressants and dietary mechanisms in an experimental disease model for necrotic enteritis. Poult Sci; 83(12):1948–1952.
4. Porter, R.E. (1998). Bacterial enteritides of poultry. Poult. Sci.; 77(8):1159–65.
5. Paiva, D. and McElroy, A. (2014). Necrotic enteritis: Applications for the poultry industry. J. Appl. Poult. Res. 23:557–566. doi.org/ 10.3382/japr. 2013-00925
6. Latinovic, V. **(**1983). Study of the characteristics of ***Clostridium perfringens*** strains isolated from broilers with enteritis. Veterinaria (Yugoslavia). **32:**267–275.
7. Dewan, M. L., and P. M. Das**. (**1989). Necrotic gastroenteritis in poultry. Bangladesh Vet. **6:**54–55.
8. Johansson, A. (2006). ***Clostridium perfringens*** the causal agent of necrotic enteritis in poultry. Doctoral thesis; Swedish University of Agricultural Sciences.
9. Tamirat, B., Tamirat, H., Bassazin, G., Alemayhu, T. and Tadesse, M. (2017). Review on Necrotic Enteritis. Brit. J. of Poult. Scie. 6 (3): 63-72. DOI: 10.5829/idosi.bjps.2017.63.72.
10. Gazdzinski, P. and Julian, R.J. (1992). Necrotic enteritis in turkeys. Avian Dis. 36:792–798.
11. Wobeser, G. and Rainnie, D.J.(1987). Epizootic necrotic enteritis in wild geese. J. Wildlife Dise. 23: 376-85.
12. Duchesnes, C., Granum, P.E., Menozzi, M.G., Peck, M., Pelkonen, S., Popoff, M., Stackebrandt, E. and Titball, R. (2006). EUROPEAN COMMISSION European Concerted Action QLK2-CT2001-01267: Clostridia in medical, veterinary and food microbiology Diagnosis and typing. Key Action 2 – Control of infectious diseases EUR 21463 EN.

13. Asaoka, Y., Yanai, T., Hirayama, H., Une, Y., Saito, E., Sakai, H., Goryo, M., Fukushi, H., and Masegi, T.(2004). Fatal necrotic enteritis associated with ***Clostridium perfringens*** in wild crows (***Corvus macrorhynchos***). Avian Pathology 33, 19-24.
14. Kwon, Y.K., Lee, Y.J., Mo, IP. 2004. An outbreak of necrotic enteritis in the ostrich farm in Korea. J. Vet.Med. Scie. 66: 1613-1615.
15. Hofshagen, M. & Stenwig, H. (1992). Toxin production by ***Clostridium perfringens*** isolated from broiler chickens and capercaillies (***Tetrao urogallus***) with and without necrotizing enteritis. Avian Diseases 36, 837-843.
16. Lovland, A., Kaldhusdal, M. (2001). Severely impaired production performance in broiler flocks with a high incidence of Clostridium perfringens-associated hepatitis. Avian Pathol; 30:73–81.
17. Cooper, K.K. and Songer, J.G. (2009). Necrotic enteritis in chickens: A paradigm of enteric infection by ***Clostridium perfringens*** type A. Anaerobe; 15: 55–60.
18. Parish, W.E. (1961a. III). The experimental disease. Journal of Comparative Pathology. 71: 405-13.
19. Parish, W.E. 1961b. Necrotic enteritis in the fowl (***Gallus gallus domesticus***). I. Histopathology of the disease and isolation of a strain of ***Clostridium welchii***. Journal of Comparative Pathology; 71: 377-93.
20. Parish, WE. 1961c. II. Examination of the causal ***Clostridium welchii***. Journal of Comparative Pathology; 71: 394-404.
21. Ficken, M.D. & Wages, D.P. 1997. Diseases of Poultry. Mosby -Wolfe. Ames, Iowa, USA. Pp; 261-264
22. Al-Sheikhly, F. and Truscott, RB. (1977a). The interaction of ***Clostridium perfringens*** and its toxins in the production of necrotic enteritis of chickens. Avian Dis. 21:256–263.
23. Al-Sheikhly,F. and Truscott, RB.(1977b). The pathology of necrotic enteritis of chickens following infusion of broth cultures of *Clostridium perfringens* into the duodenum. Avian Dis. 21:230–240.
24. Niilo, L. (1978). Enterotoxigenic ***Clostridium perfringens*** type A isolated from intestinal contents of cattle, sheep, and chickens. Can. J. Comp. Med. 42:357–363.
25. Nairn, M.E. and Bamford, V.W.(1967). Necrotic enteritis of broiler chickens in western Australia. Aust Vet J. 43:49–54.
26. Shane, S.M., Gyimah, J.E., Harrington, K.S., Snider, T.G. (1985). Etiology and pathogenesis of necrotic enteritis. Vet. Res. Commun. 9:269–287.
27. Popoff, MR., Milward, F.W., Bancillon, B., Boquet, P.(1989). Purification of the ***Clostridium spiroforme*** binary toxin and activity of the toxin on HEp-2 cells. Infect. Immun. 57:2462–2469.
28. Köhler, B., Marx, G., Kolbach, S., and Böttcher, E. (1974). Untersuchungen zur nekrotischen enteritis der Hu¨hner. 1. Diagnostik und Bekam- pfung. Monatsh. Veterinaermed. **29:**380–384.
29. Long, J.R. and Truscott, R.B.(1976). Necrotic enteritis on broiler chickens. III. Reproduction of the disease. Can. J. Comp. Med. 40:53–59.

30. Hamdy, A.H., Thomas, R.W., Kratzer, D.D., Davis, R.B. (1983a). Lincomycin dose response for treatment of necrotic enteritis in broilers. Poult Sci; 62(4):585–8.
31. Hamdy, A.H., Thoma,s R.W., Yancey, R.J., Davis, R.B. (1983b). Therapeutic effect of optimal lincomycin concentration in drinking water on necrotic enteritis in broilers. Poult. Sci. 62:589–591.
32. Songer, J.G. (1996). Clostridial enteric diseases of domestic animals. Clin. Microbiol. Rev.; 9:216-234.
33. Brennan, J., Skinner, J., Barnum, D.A., Wilson, J. (2003). The efficacy of bacitracin methylene disalicylate when fed in combination with narasin in the management of necrotic enteritis in broiler chickens. Poult Sci;82(3):360–3.
34. Lovland, A., Kaldhusdal, M., Redhead, K., Skjerve, E., Lillehaug, A. (2004). Maternal vaccination against subclinical necrotic enteritis in broilers. Avian Pathol; 33(1):83–92.
35. Thompson, D.R., Parreira, V.R., Kulkarni, R.R., Prescott, J.F.(2006). Live attenuated vaccine-based control of necrotic enteritis of broiler chickens. Vet Microbiol; 113(1–2):25–34.
36. Kiu, R. and Hall, L.J. (2018). An update on the human and animal enteric pathogen *Clostridium perfringens*. Emerging Microbes & Infections 7:141. DOI 10.1038/s41426-018-0144-8.
37. Dosoky, R.M. (1989). The role of environment in the occurrence of clostridial infection among fowl. Assiut Vet. Med. J. 24:165–171.
38. Truscott, R.B, and Al-Sheikhly, F. (1977). Reproduction and treatment of necrotic enteritis in broilers. Am. J. Vet. Res. 38:857–861.
39. Al-Sheikhly, F. and Al-Saieg, A. (1980). Role of coccidia in the occurrence of necrotic enteritis of chickens. Avian Dis. 24:324–333.
40. Al-Sheikhly, F.and Truscott, R.B. (1977c). The pathology of necrotic enteritis of chickens following infusion of crude toxins of ***Clostridium perfringens*** into the duodenum. *Avian Dis.* **21**, 241-255.
41. El-seedy, F.R. (1990). Studies on necrotic enteritis in chickens. Veterinary Medical Journal, 38: 407-417.
42. Mc Orist, S., and Reece, R.L. (1992). Clostridial enteritis in free-living lorikeets (***Trichoglossus*** spp.). Avian Pathol. 21:503–507.
43. Gholamian-dekhordi, A.R., Ducatelle, R., Heyndrickx, M., Haesebrouck, F., Van Immerseel, F.(2006). Molecular and phenotypical characterization of ***Clostridium perfringens*** isolates from poultry flocks with different disease status. Vet Microbiol; 113(1–2):143–52.
44. Nadas, U.G., Berkoglu, A., and Alp, R.(1991). Detection of ***Clostridium perfringens*** alpha antitoxin in the serum of healthy fowls and determination of its relationship with enterotoxaemia. Pendik Hayvan Hastaliklari Merkez Arastirma Enstitusu Dergisi 22:94–106.
45. Keyburn, A.L., Boyce, J.D., Vaz, P., Bannam, T.L., Ford, M.E., Parker, D. (2008). NetB a new toxin that is associated with avian necrotic enteritis caused by ***Clostridium***

perfringens. PLoS Pathog; 4(2): e26.

46. Mostafa, A.H., Abdeen, E.E., and Abou-Hadeed, M.G. (2016). Multiplex PCR and Detection of netB Gene of *Clostridium perfringens* from Broilers with Necrotic Enteritis. Asian Journal of Animal and Veterinary Advances, 11:284-252. DOI: 10.3923/ajava.2016. 248. 252.
47. Ronco, T., Stegger, M., Ng, K.L., Lilje, B., Lyhs, U., Andersen, P.S., and Pedersen, K. (2017). Genome analysis of *Clostridium perfringens* isolates from healthy and necrotic enteritis infected chickens and turkeys. BMC Res Notes 10:270. DOI 10.1186/s13104-017-2594-9.
48. Mwangi, S., Timmons, J., Fitz-coy, S., and Parveen, S. (2018). Characterization of *Clostridium perfringens* recovered from broiler chicken affected by necrotic enteritis. Poultry Science 0:1–8. http://dx.doi.org/10.3382/ps/pey 332.
49. Quinn, P.J., Carter, M.E., Markey, B., Carter, G.R. (2004). Clinical Veterinary Microbiology. 6th ed. Edinburgh, New York: Mosby. Pp. 191-208.
50. Quinn, P.J., Markey B.K., Leonard F.C., Fitz Patric E.S., Fanning S. and Hartigan P.J. (2011). Veterinary Microbiology & Microbial disease. 2nd ed, UK, Wiley-Blackwell, USA. PP: 243-247.
51. Adams, C. (2004). Nutricines in poultry production: focus on bioactive feed ingredients. Nutr Abst Rev Ser B; 74:1–12.
52. Cato, E. P., George, W. L. & Finegold, S. M. (1986). Genus Clostridium Prazmowski 1880, 23AL. In Bergey's Manual of Systematic Bacteriology, vol. 2, pp. 1141-1200.
53. Yoo, H.S., Lee, S.U., Park, K.Y., Park, Y.H.(1997). Molecular typing and epidemiological survey of prevalence of ***Clostridium perfringens*** types by multiplex PCR. J Clin Microbiol; 35(1):228–32.
54. Li, J., Freedman, J.C., Evans ,D.R. & McClane, B.A. (2017). CodY promotes sporulation and enterotoxin production by *Clostridium perfringens* type A strain SM101. Infect. Immun. 85, https://doi.org/10.1128/IAI.00855-16.
55. Prescott, J.F., Smyth, J.A., Shojadoost, B. & Vince, A. (2016). Experimental reproduction of necrotic enteritis in chickens: a review, Avian Pathology, 45(3): 317-322. DOI: 10.1080/03079457.2016.1141345.
56. Williams RB, Marshall RN, La Ragione RM, Catchpole J. 2003.A new method for the experimental production of necrotic enteritis and its use for studies on the relationships between necrotic enteritis, coccidiosis and anticoccidial vaccination of chickens. Parasitol Res; 90(1):19–26.
57. Baba, E., Yasuda, N., Fukata, T., Arakawa, A. (1988). Effect of Eimeria tenella infection on the caecal population of lincomycin-resistant ***Clostridium perfringens*** introduced into chickens. Res Vet Sci;45(2):219–21.
58. Baba, E., Fuller, A.L., Gilbert, J.M., Thayer, S.G., McDougald, L.R. (1992). Effects of ***Eimeria brunetti*** infection and dietary zinc on experimental induction of necrotic

enteritis in broiler chickens. Avian Dis;36(1):59–62.

59. Baba, E., Ikemoto, T., Fukata, T., Sasai, K., Arakawa, A., McDougald, L.R. (1997). Clostridial population and the intestinal lesions in chickens infected with ***Clostridium perfringens*** and ***Eimeria necatrix***. Vet Microbiol;54(3–4):301–8.
60. Drew, M.D., Syed, N.A., Goldade, B.G., Laarveld, B., Van Kessel, A.G. (2004). Effects of dietary protein source and level on intestinal populations of ***Clostridium perfringens*** in broiler chickens. Poult Sci; 83(3):414–20.
61. Branton, S.L., Reece, F.N., and Hagler, W.M. (1987). Influence of a wheat diet on mortality of broiler chickens associated with necrotic enteritis. Poult. Sci.; 66:1326–1330.
62. Kaldhusdal, M. (1994). The occurrence of necrotic enteritis in slaughtered fowls varies with the composition of their feed. Norsk Veterinaertidsskr; 106: 309.
63. Kaldhusdal, M. and Hofshagen, M. (1992). Barley inclusion and avoparcin supplementation in broiler diets. 2. Clinical, pathological and bacteriological findings in a mild form of necrotic enteritis. Poult. Sci.; **71**: 1145-1153.
64. Antonissen, G., Eeckhaut,. V, Van Driessche, K., Onrust, L., Haesebrouck, F., Ducatelle, R., Moore, R.J. & Van Immerseel, F. (2016). Microbial shifts associated with necrotic enteritis. Avian Pathology, 45(3): 308-312. DOI:10.1080/ 03079457.2016.1152625.
65. Kaldhusdal, M., Schneitz, C., Hofshagen, M., Skjerve, E. (2001). Reduced incidence of Clostridium perfringens-associated lesions and improved performance in broiler chickens treated with normal intestinal bacteria from adult fowl. Avian Dis; 45(1):149–56.
66. Branton, S.L., Lott, B.D., Deaton, J.W., Maslin, W.R., Austin, F.W., Pote, L.M. (1997). The effect of added complex carbohydrates or added dietary fiber on necrotic enteritis lesions in broiler chickens. Poult Sci;76(1):24–8
67. Smyth, J.A. (2016). Pathology and diagnosis of necrotic enteritis: is it clear-cut? Avian Pathology, 45(3): 282-287. DOI: 10.1080/03079457.2016. 1158780
68. Van Immerseel, F., De Buck, J., Pasmans, F., Huyghebaert, G., Haesebrouck, F., Ducatelle, R. (2004). ***Clostridium perfringens*** in poultry: an emerging threat for animal and public health. Avian Pathol; 33(6):537–49.
69. Baba, E., Wakeshima, H., Fukui, K., Fukata, T., Arakawa, A. (1992). Adhesion of bacteria to the cecal mucosal surface of conventional and germ-free chickens infected with ***Eimeria tenella***. Am J Vet Res;53(2):194–7.
70. Li, J., Paredes-Sabja, D., Sarker, M.R. & McClane, B.A. (2016). *Clostridium perfringens* sporulation and sporulation-associated toxin production. Microbiol. Spectr. 4, https://doi.org/10.1128/microbiolspec.TBS-0022-2015.
71. Fukata, T., Hadate, Y., Baba, E., and Arakawa, A. (1991). Influence of bacteria on ***Clostridium perfringens*** infections in young chickens. Avian Dis. 35:224– 247.
72. Fukata, T., Hadate, Y., Baba, E., Uemura, T., Arakawa, A. (1988). Influence of ***Clostridium***

perfringens and its toxin in germ-free chickens. Res Vet Sci; 44(1):68–70.

73. Fossum, O., Sandstedt, K., and Engström BE. (1988). Gizzard erosions as a cause of mortality in white leghorn chickens. Avian Pathol. **17**: 519-525.
74. Craven, S.E., Cox, N.A., Bailey, J.S., Cosby, D.E. (2003). Incidence and tracking of Clostridium perfringens through an integrated broiler chicken operation. Avian Dis; 47(3):707–11.
75. Long, J.R., Petit, J.R., Barnum, D.A. (1974). Necrotic enteritis in broiler chickens. II. Pathology and proposed pathogenesis. Can. J. Comp. Med. Vet. Sci. **38**, 467-474.
76. Shane, S.M. (2005). Handbook on poultry diseases. 2nd ed., American Soybean Association. Printed in Singapore. PP: 138-140.
77. Helmboldt, C. F., and Bryant, E. S. 1971. The pathology of necrotic enteritis in domestic fowl. Avian Dis. 15:775–780.
78. Brennan, J., Bagg, R., Barnum, D., Wilson, J., Dick, P. (2001a). Efficacy of narasin in the prevention of necrotic enteritis in broiler chickens. Avian Dis;45(1):210–4.
79. Craven, S.E., Stern, N.J., Cox, N.A., Bailey, J.S., Berrang, M. (1999). Cecal carriage of ***Clostridium perfringens*** in broiler chickens given Mucosal Starter Culture. Avian Dis: 43(3):484–90.
80. Broussard, C.T., Hofacre, C.L., Page, R.K., Fletcher ,O.J. (1986). Necrotic enteritis in cage-reared commercial layer pullets. Avian Dis. Jul-Sep; 30(3): 617-9.
81. Long, J.R., Petit, J.R., Barnum, D.A. (1974). Necrotic enteritis in broiler chickens. II. Pathology and proposed pathogenesis. Can. J. Comp. Med. Vet. Sci. **38**, 467-474.
82. Lovland, A., Kaldhusdal, M. (1999). Liver lesions seen at slaughter as an indicator of necrotic enteritis in broiler flocks. FEMS Immunol Med Microbiol; 24(3):345–51.
83. Da Costa, F., Mot, D., Bokori-Brown, M., Savva, G., Basak,K., Van Immerseel, F. and Titball,W. (2013). Protection against avian necrotic enteritis after immunization with NetB genetic or formaldehyde toxoids. Vaccine, 31(37): 4003-4008.
84. Tille, P.M. (2017). Bailey & Scott's Diagnostic Microbiology. 14th ed, Elsevier, St. Louis, Missouri 63043. Pp; 502-505.
85. Cappuccino, J.G. and Welsh, C. (2018). Microbiology A laboratory manual. 11th ed., Pearson Education Limited. Harlow, England. Pp; 141-145.
86. Songer, J.G. and Post K.W. (2005). Veterinary Microbiology: Bacterial & fungal Agents of Animal Disease. Philadelphia, Elsevier Sunders. PP: 178-179.
87. Carter, G.R. and Wise, D.J. (2004). Essentials of veterinary bacteriology and mycology; 6th ed., Ames, Iowa - Iowa State Press, Pp; 122-124.
88. Naylor, R.D., Martin, P.K., Sharpe, R.T. (1987). Detection of Clostridium perfringens epsilon toxin by ELISA. Res Vet Sci. 42:255-256.
89. El Idrissi A.H., Ward, G.E. (1992). Evaluation of enzyme-linked immunosorbent assay for diagnosis of Clostridium perfringens enterotoxemias. Vet Microbiol. 31: 389-396.
90. Hamad, M.A., Habra, N., Kalb-Allouz A. (2009). Diagnosis of

Clostridium perfringens strains isolated from enterotoxemia cases in sheep using polymerase chain reaction (PCR) technique. Iraqi Journal of Veterinary Sciences, 23(1): 229-232.

91. Hamad, M.A., Habra, N., Kalb-Allouz, A. (2010). Biotyping of Clostridium Perfringens strains isolated from enterotoxemia cases in sheep using ELISA technique. Iraqi Journal of Veterinary Sciences, 24(1): 17-22.
92. Kumar, N.V., Sreenivasulu, D. and Reddy, Y.N. (2016). Identification of *Clostridium Perfringens* Type C and D from Enterotoxemia Suspected Lambs in Andhra Pradesh. Indian Vet. J., 93 (1) : 29 – 31.
93. Gokce, H.I., Genc, O., Sozmen, M., Gokce, G. (2007). Determination of Clostridium perfringens Toxin-Types in Sheep with Suspected Enterotoxemia in Kars Province, Turkey. Turk J Vet Anim Sci. 31(5):355-360.
94. Brennan, J., Moore, G., Poe, S.E., Zimmermann, A., Vessie, G., Barnum, D.A. (2001b). Efficacy of in-feed tylosin phosphate for the treatment of necrotic enteritis in broiler chickens. Poult Sci;80(10):1451–4.
95. Takeda, T., Fukata, T., Miyamoto, T., Sasai, K., Baba, E., Arakawa, A. (1995). The effects of dietary lactose and rye on cecal colonization of ***Clostridium perfringens*** in chicks. Avian Dis; 39(2):375–81.
96. Shane, S.M., Koetting, D.G., Harrington, K.S. (1984). The occurrence of ***Clostridium perfringens*** in the intestine of chicks. Avian Dis. 28:1120–1124.
97. Kaldhusdal, M. and Skjerve, E.(1996). Association between cereal contents in the diet and incidence of necrotic enteritis in broiler chickens in Norway. Prev Vet Med; 28:1–16.
98. Wages, D.P. and Opengart, K. (2003). Necrotic Enteritis. In: Diseases of Poultry, 11th ed. (Saif Y.M., Ed.). Iowa State Press, Iowa, USA. Pp; 781-785,